Appl. No.: 09/424,244 Atty. Dkt. No. P64075US0

Bunt

wavelength of 405 nm. The assays were performed in microtitration plates with or without a glass bottom.

At page 22, replace the fifth full paragraph (beginning at line12) with the following paragraph.

3

The cells were sedimented by centrifugation, the culture supernatants were taken off and, if necessary, stored on ice. Assays were performed in microtitration plates without a glass bottom. The lipase assay buffer ($10\,\text{mM}\,\text{CaCl}_2$, $0.1\%\,\text{TRITON}$ X-100 and 20 mM Tris/HCl, pH 8.5) contained the chromogenic lipase substrate pnitrophenyl caprylate [Sigma] in a concentration of 5 mM. Per measuring sample, 5 μ l each of the culture supernatants was mixed with 95 μ l lipase assay buffer. The conversation of the substrate was determined by photometry using a microtitration plate (ELISA) reader.

IN THE CLAIMS

Cancel claims 1-14, without prejudice or disclaimer, and add the following claims.

- 15. A method for identifying active substances which affect the covalent bonding of polypeptides to the surface of Gram-positive bacteria, comprising the following steps:
 - a) providing a sample of Gram-positive bacteria which contain or produce at least one enzymatic reporter substances which is or can become covalently bonded to the surface of the Gram-positive bacteria, said at least one reporter substance having a

Appl. No.: 09/424,244 Atty. Dkt. No. P64075US0

different enzymatic activity when not covalently bonded to the surface of the Grampositive bacteria from that exhibited when it is covalently bonded to the surface of the Gram-positive bacteria;

- b) contacting the sample with a possible active substance;
- c) assaying the enzymatic activity of the reporter substance of the Gram-positive bacteria of the sample; and
- d) correlating the enzymatic activity of the reporter substance to a capability of the active substance to affect the covalent bonding of polypeptides to the surface of gram-positive bacteria.

The method according to claim 15, characterized in that said assaying of the emzymatic activity of the reporter substance is done by comparison with at least one reference sample which has not been genetically altered, and/or at least one reference sample in which the reporter substance is non-covalently bonded to the surface of Gram-positive bacteria, and/or at least one reference sample in which the reporter substance is covalently bonded to the surface of the Gram-positive bacteria, and/or at least one reference sample in which the reporter substance is present without covalent bonding to the surface of the Gram-positive bacterial.

17. The method according to claim 15, characterized in that said covalent bonding of the polypeptides is effected to the murein of the cell wall.

Appl. No.: 09/424,244 Atty. Dkt. No. P64075US0

- 18. The method according to claim 17, characterized in that said covalent bonding of the polypeptides is effected to the murein of the cell wall at pentaglycine interpeptide bridges.
- 19. The method according to claim 15, characterized in that said polypeptides are pathogenicity factors of Gram-positive bacterial.
- 20. The method according to characterized in that said reporter substance is a hybrid polypeptide.
- 21. The method according to claim 20, characterized in that said hybrid polypeptide has a succession of the following sequence segments: N-terminal signal peptide, enzyme, sequence segment having the sequence LPXTG, hydrophobic sequence segment, and charged sequence segment.
- 22. The method according to claim 21, characterized in that said enzyme is provided as a proenzyme.
- 23. The method according to claim 21, characterized in that said change of enzymatic activity is due to a transition of the enzyme from an inactive to an active conformation or vice versa.
- 24. The method according to claim 21, characterized in that a linker peptide, especially one comprising less than 10 amino acids, is provided between said enzyme and said sequence segment having the sequence LPXTG.
- 25. The method according to claim 15, characterized in that said Gram-positive bacteria have a low natural cell wall turnover and/or a small number of cell wall proteases and/or a small number of secreted proteases.